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Jenkins, Samantha Gini; Fonseca, César; Cannella, David; Varrone, Cristiano

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CHARACTERISING PET DEGRADING *IDEONELLA SAKAIENSIS* & ENGINEERING OF PETase THROUGH MUTAGENESIS

Samantha Jenkins, Cesar Simones da Fonseca, David Cannella, Cristiano Varrone

Universite Libre de Bruxelles and Aalborg University

Email. Sjenki17@student.aau.dk, csf@bio.aau.dk, David.cannella@ulb.ac.be, Cva@bio.aau.dk.

INTRODUCTION

8300 million tonnes of plastic produced since early 20th century. Plastic pollution leads to toxic substances being released into our oceans, land and even air. Methods such as recycling and depolymerisation whilst excellent will only ever result in more plastic.

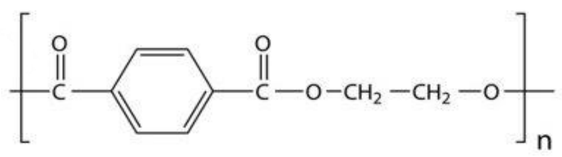
- 33% used once, and thrown away. Only 8% recycled *(Wang et al., 2015).*
- 8 million tonnes p/y released into oceans

Degrade into microplastics

- Enter the food chain and are on the rise
- Unexamined health impacts

PET (POLYETHELYENE TEREPHTHALATE)

- Crystalline plastic
- Drinks bottles, fleece, carpets
- Low melting temp
- Easy reformation
- Only 7% recycled into new products
- Made from ethylene monomers and terephthalic acid



BIODEGRADATION

- Plastic degrading microorg anisms exist in abundance (see table), the process is just too slow
- Biodegradation removes plastic from cycle and can turn plastic waste into a resource
- and result in profitable products (product engineering)

Plastic	Mircoorganism	Enzyme	Source	Reference
Polyethylene	*Brevibacillus borstelensis*		Bacterial	[35] [14]
	Rhodococcus rubber		Bacterial	[36] [37] [14]
	Penicillium simplicissimum YK		Bacterial	[38] [14]
	Ideonella sakaiensis	lipase	Bacterial	[6]
	Pseudomonas putida	peroxidase	Bacterial	[6]
Polyethylene adipate (PEA)	*Penicillium, Rhizopus arrizus*	lipase	Bacterial	[6]
Polyhydroxyalkanoate (PHA)	*Pseudomonas stutzeri*	Serine hydrolase	Bacterial	[6]
Polyethylene Terephthalate	*Ideonella sakaiensis*	Hydrolase	Bacterial	[6]
	pestalotiopsis microspora	Hydrolase	Fungal	[39]
Polyurethane	*Comamonas acidovorans TB-35*	esterase	Bacterial	[40] [41] [14]
	Pestalotiopsis microspora	Hydrolase	Fungal	[39]
	Phanerochaete chrysosporium	Manganese peroxidase	Fungal	
	Pseudomonas chlororaphis		Bacterial	[14]
	Curvularia senegalensis		Fungal	[42] [14]
	Fusarium solani		Fungal	[14]
	Cladosporium sp.		Fungal	[14]
Polyvinyl chloride	*Pseudomonas putida AJ*		Bacterial	[14]
	Ochrobactrum TD		Bacterial	[14]
	Pseudomonas fluorescens B-22		Bacterial	[43] [14]
	Aspergillus niger		Bacterial	[14]
Polycaprolactone (PCL)	*Aspergillus flavus*	Glucosidase	Fungal	[6]
	Aspergillus niger		Fungal	[14]
	Fusarium		Fungal	[14]
	Rhizopus delemar		Bacterial	[14]
	Penicillium, Rhizopus arrizus	Lipase	Bacterial	[6]
Polybutylene succinate (PBS)	*Aspergillus oryzae*	cutinase	Fungal	[6]
	Penicillium, Rhizopus arrizus	Lipase	Bacterial	[6]

IDEONELLA SAKAIENSIS

Most effective plastic (PET) degrading organism. *(Oda group, Kyoto Institute of Technology, 2016)*

Gram negative, aerobic, rod-shaped, non-spore forming, single flagellated

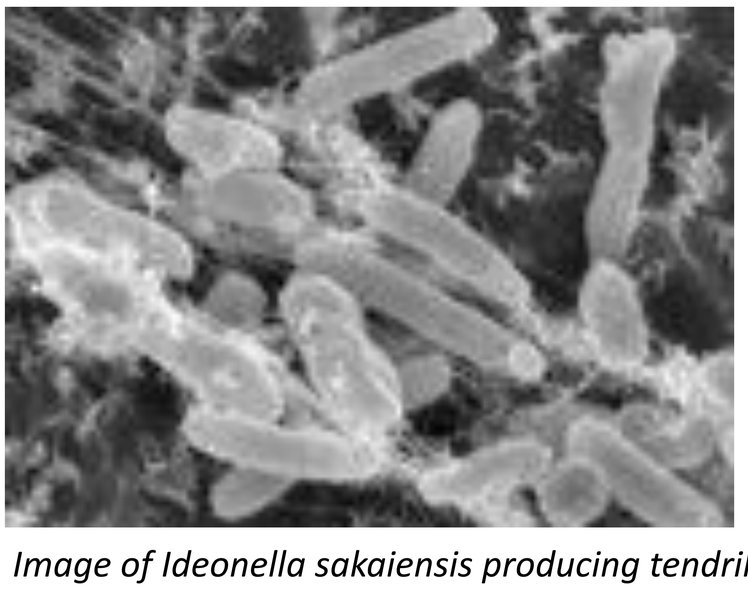


Image of Ideonella sakaiensis producing tendrils which attach to substrate. (Yoshida et al., 2016)

PROJECT AIM

Characterise the bacterial strain *Ideonella sakaiensis* for the engineering of it's plastic degrading PETase enzyme to increase its catalytic activity using genetic engineering techniques such as directed mutagenesis.

MATERIALS AND METHODS

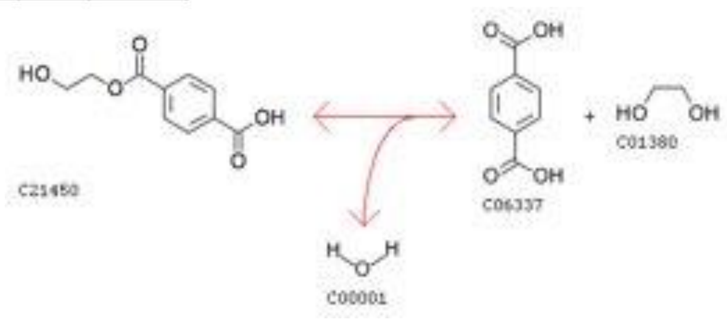
CHARACTERISING *I. SAKAIENSIS*

Preference to grow on solid medium with PET or PE plastic as attachment of tendrils to substrate is require to begin degradation:

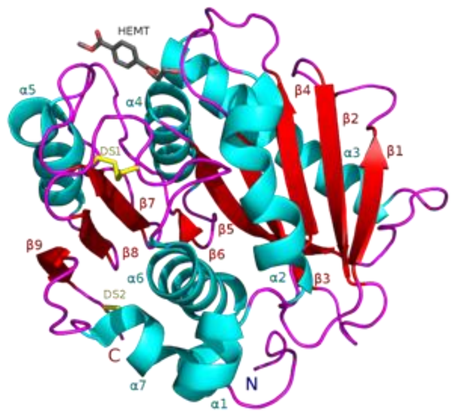
Examples of I.Sakaiensis Colonies; round Clear and small

ENZYME

- Serine hydrolase, charge relay system
- Coupled with MHETase which breaksdown the products further in ethelyene glycol and terepthalic acid
- PETase bottleneck of enzyme cascade



Degradation of PET into Ethelyene glycol and terepthalic acid by PETase



3D structure of PETase enzyme, (Han et al., 2017)

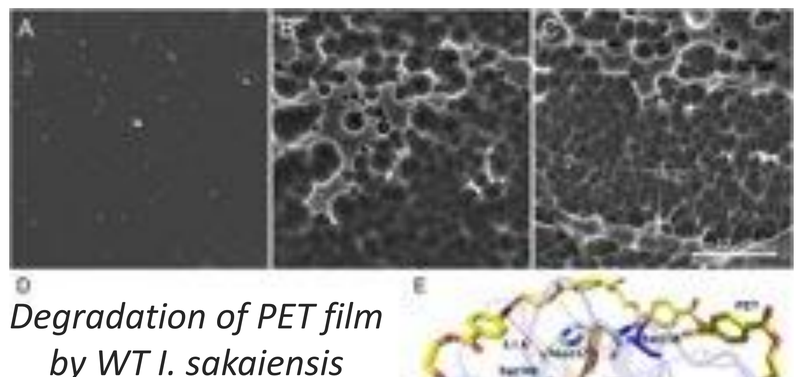
DIRECTED MUTAGENESIS

Modifications to enzyme active site improve binding of substrate:

S238F & W159H mutation:

- Serine to phenalalynine (S238F) = more aromatic interactions & stable 'docking' for PET
- Wild-type PET stabilised by W185 and W159 interactions only.

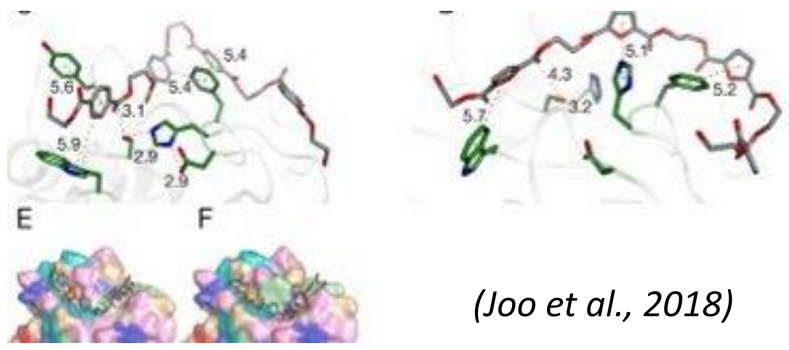
Double mutant provides four aromatic interactions to stabilise the PET



Degradation of PET film by WT I. sakaiensis (left) and double mutant (far right) (Austin et al., 2018)

Arg280 mutation:

- Residue in the binding site (Arg280) = polar and protruding region
- Substituting Arg280 for Alanine, = longer, unhindered, substrate binding.



(Joo et al., 2018)

FUTURE PERSPECTIVES

Faster and more broad spectrum plastic biodegradation

Make plastic into a resource by engineering products

Bio-sensors: Ocean microplastic problem

Demobilization onto filters

Demobilization onto underwater structures (artificial reefs)

Synthetic consortia

References:

Austin, H.P., Allen, M.D., Donohoe, B.S., Rorrer, N.A., Kearns, F.L., Silveira, R.L., Pollard, B.C., Dominick, G., Duman, R., El Omari, K. and Mykhaylyk, V., 2018. Characterization and engineering of a plastic-degrading aromatic polyesterase. *Proceedings of the National Academy of Sciences*, *115*(19), pp.E4350-E4357

Champakalakshmi Ravi Kyaw Myint Bhone, Sing Lim Chu Sakharkar Meena Kishore, and Sakharkar Kishore R. Biodegradation of Low Density Polythene (LDPE) by Pseudomona Species. Rivista degli infortuni e delle malattie professionali, 1951

Han, X., Liu, W., Huang, J.W., Ma, J., Zheng, Y., Ko, T.P., Xu, L., Cheng, Y.S., Chen, C.C. and Guo, R.T., 2017. Structural insight into catalytic mechanism of PET hydrolase. *Nature communications*, *8*(1), p.2106.

Joo, S., Cho, I.J., Seo, H., Son, H.F., Sagong, H.Y., Shin, T.J., Choi, S.Y., Lee, S.Y. and Kim, K.J., 2018. Structural insight into molecular mechanism of poly (ethylene terephthalate) degradation. *Nature communications*, *9*(1), p.382.

Liu, B., He, L., Wang, L., Li, T., Li, C., Liu, H., Luo, Y. and Bao, R., 2018. Protein Crystallography and Site-Direct Mutagenesis Analysis of the Poly (ethylene terephthalate) Hydrolase PETase from Ideonella sakaiensis. *ChemBioChem*, *19*(14), pp.1471-1475.

Obranić, S., Babić, F. and Maravić-Vlahoviček, G., 2013. Improvement of pBBR1MCS plasmids, a very useful series of broad-host-range cloning vectors. Plasmid, 70(2), pp.263-267.

Tanasupawat, S., Takehana, T., Yoshida, S., Hiraga, K. and Oda, K., 2016. Ideonella sakaiensis sp. nov., isolated from a microbial consortium that degrades poly (ethylene terephthalate). *International journal of systematic and evolutionary microbiology*, *66*(8), pp.2813-2818.

Yoshida, S., Hiraga, K., Takehana, T., Taniguchi, I., Yamaji, H., Maeda, Y., Toyohara, K., Miyamoto, K., Kimura, Y. and Oda, K., 2016. A bacterium that degrades and assimilates poly (ethylene terephthalate). *Science*, *351*(6278), pp.1196-1199.

Yutaka Tokiwa, Buenaventurada Calabia, Charles Ugwu, Seiichi Aiba, Yutaka Tokiwa, Buenaventurada P. Calabia, Charles U. Ugwu, and Seiichi Aiba. Biodegradability of Plastics. International Journal of Molecular Sciences, 10(9):3722–3742, 8 2009